Optimizing Flashlamp Target Activated Microdissection (fTAM) for Clinical Pathology

Olivia Tran, Robert Bonner, Ph.D.[1], Tom Pohida[2], Randall Pursley[2], Nicole Morgan, Ph.D.[3], Michael Kane[2], John Kakareka[2]

[1]Section of Medical Biophysics, NICHD, [2]Signal Processing and Instrumentation Section, DCB/CIT, [3]Biomedical Engineering and Physical Science Shared Resource, NIBIB

Background

- Molecular (DNA, RNA, protein) analysis of cancers increasingly is required to choose the best treatment option. Laser Capture Microdissection (LCM) allows the isolation of highly purified target cell populations from a tissue section for downstream comprehensive molecular analysis. However the cost and complexity of LCM has limited its routine clinical utility.[1]
- Target-expression activated Microdissection (xMD/TAM) uses the absorption of specific tissue stains to generate the heat required to bond the EVA thermoplastic tape. This allows the whole slide to be illuminated but captures only the stained targets on the transfer film. Without using a microscope or user targeting, TAM greatly simplifies cell targeting and increases its speed without sacrifice of precision and specificity.[3,4]

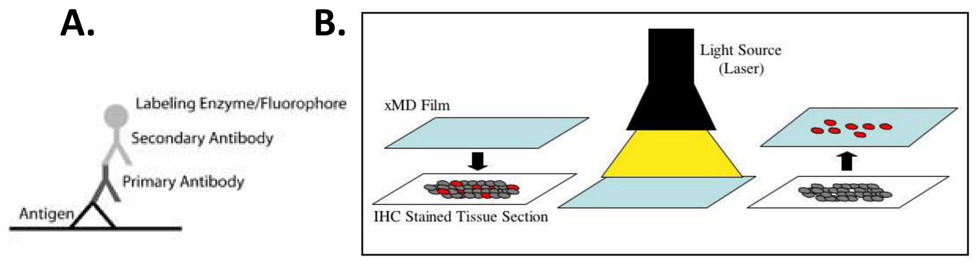


Figure 1 (A) Immunohistochemistry staining.[4]
(B) Schematic representation of xMD technology.[3]

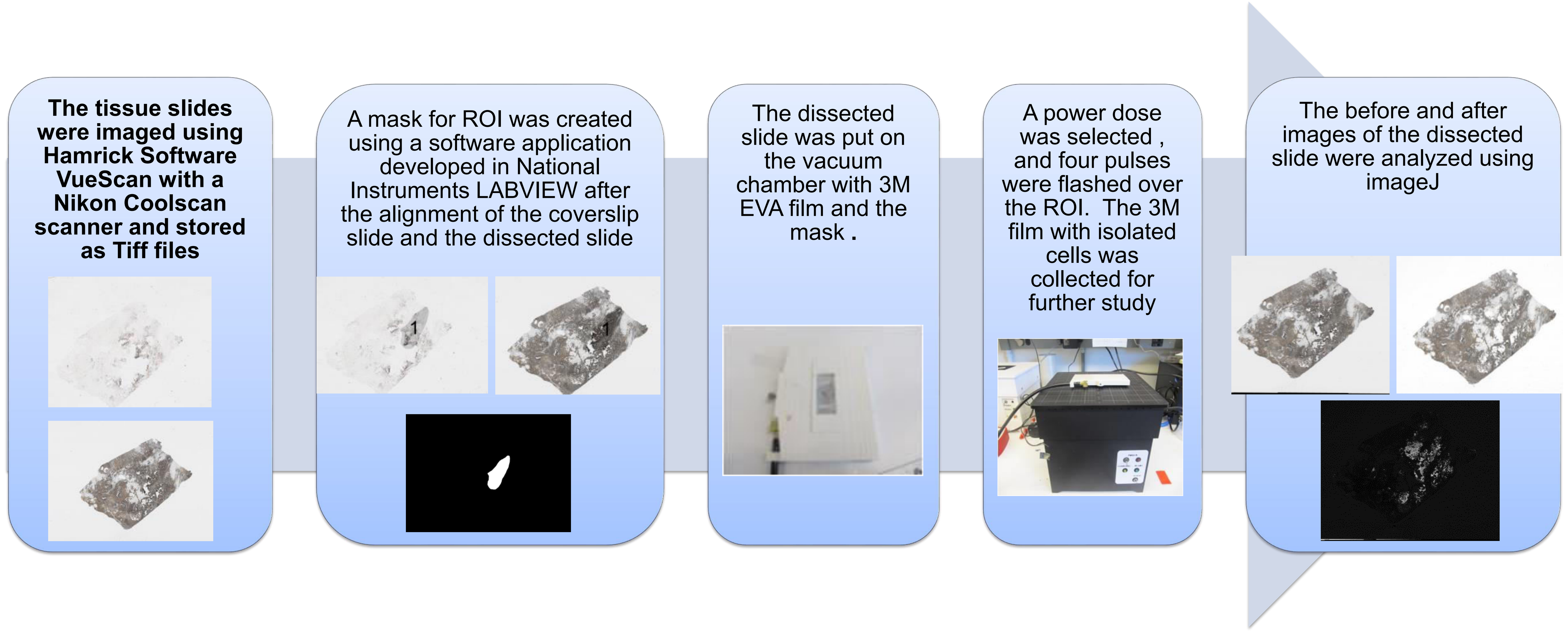
- Our initial computer controlled laser-scanning TAM system allowed selection of regions of interest and dosimetry arrays to optimize specific capture on a given slide with a variety of non-commercial transfer film.
- We have recently developed a much simpler, low-cost flashlamp TAM system (fTAM) which a number of research centers have asked to use for rapid isolation of specifically immunostained targets within formalin-fixed paraffin embedded [FFPE] tissue sections.
- What are the requirements for remote site use of fTAM beta systems that we can reasonably supply before commercialization?

Objectives

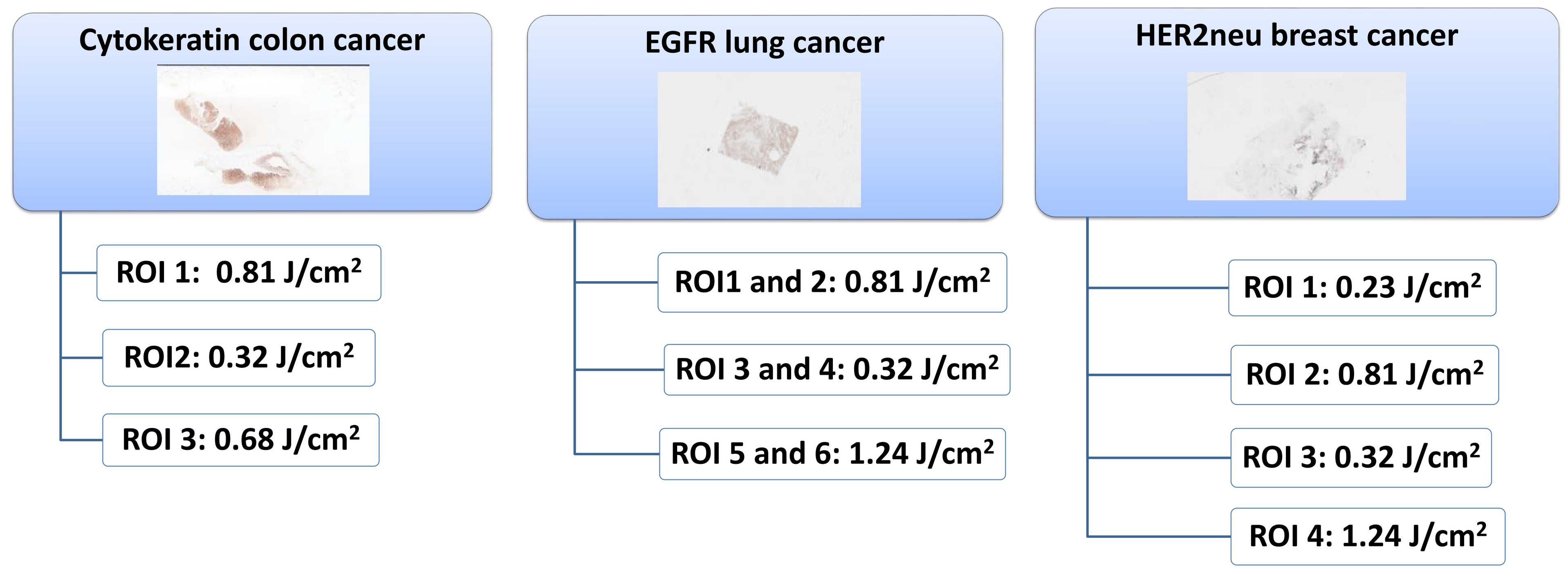
Develop a simple method for routine use of fTAM prototypes at beta test sites.

1. Establish protocol for commercial 3M EVA tape in fTAM of FFPE clinical cancer tissue sections.
2. Develop method to create user-defined reflective tape masks to limit flashlamp illumination to specific regions of interest ($\geq$0.5mm^2),
3. Study effects of flashlamp light dose on efficiency and specificity of microcapture for immunostained targets of varying optical density and spatial distribution.
4. Evaluate nuclear capture efficiency for three specific immunostained cellular targets (nucleus [Ki67]; cytoplasm [pan cytokeratin]; cell membrane [EGFR]).

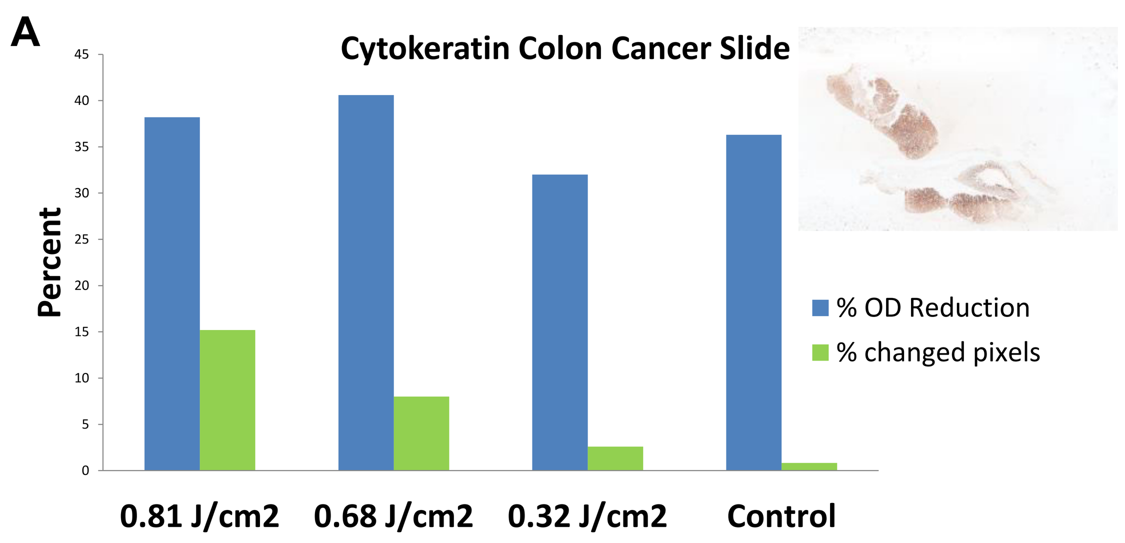
Procedure

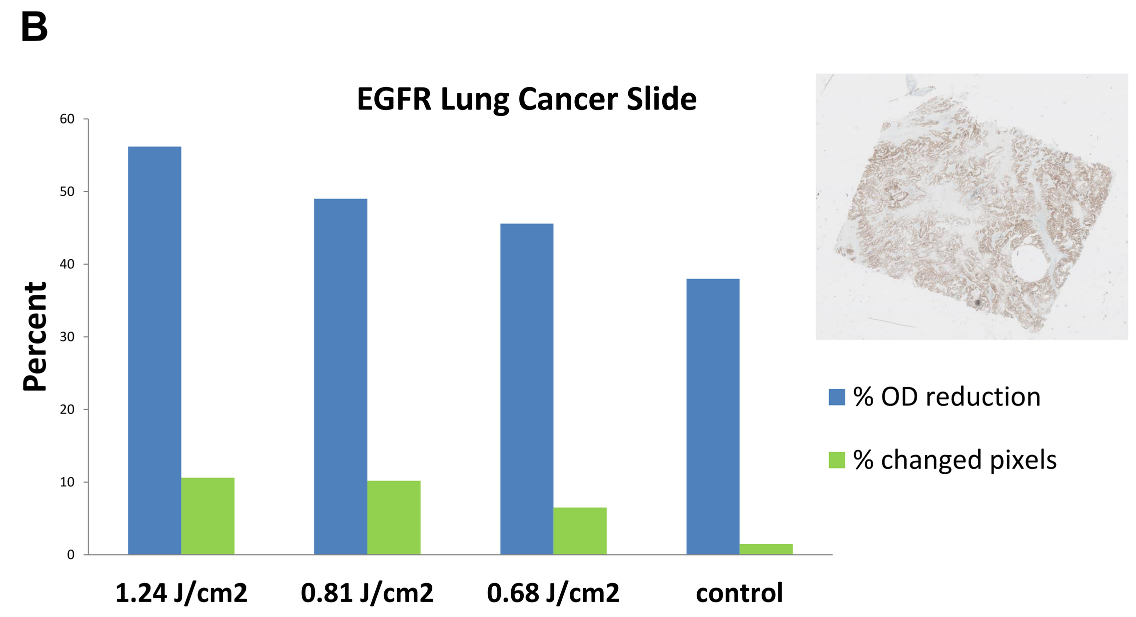


The procedure was repeated for each ROI treated with different energy dose on cytokeratin, EGFR and HER2neu tissue slides.



Results: Capture Efficiency





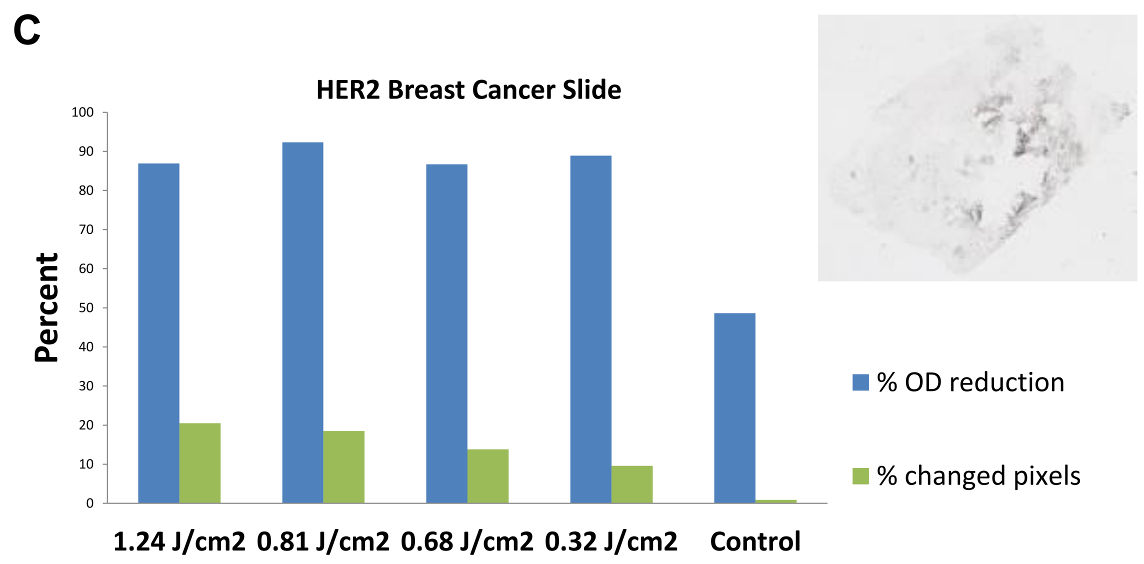


Figure 2. Image analysis using ImageJ after fTAM. Average efficiency of capture for different flashlamp light doses on EGFR, HER2neu and Cytokeratin tissue slides. Percent reduction stain intensity of target pixels and the fraction of pixels captured determined by analysis of before and after images using ImageJ. %OD reduction data includes only those pixels with intensity change above a user-determined threshold.

A: Cytokeratin slide , there are significant decreasing of percent changed pixels as power is decreased. The control percent changed pixels is 0.8%, and the percent OD reduction is 36 %.
B: EGFR slide, there are more pixels that reduced OD as the power is increased, the control is 1.6% changed pixels with 38% OD reduction.
C: HER2neu slide, the percent changed pixels is proportional to the energy dose , but the percent OD reduction is not consistent. The control is 0.9% changed pixels with 49% OD reduction.

Very few pixels change in the control region. The large fractional change observed in these few pixels is due to mis-registration by a few pixels of lumen edges in the before and after images which give a high fractional change.

Results: Images

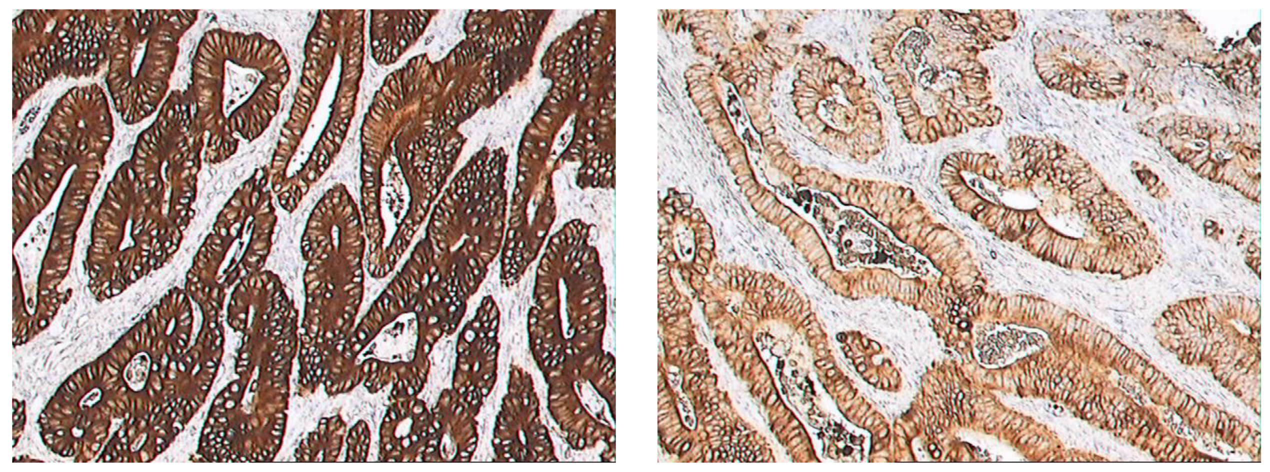


Figure 3. Cytokeratin stained colon cancer, 40x images, no coverslip. Left: as received; Right: after fTAM (ROI 2 - 0.32J/cm^2)

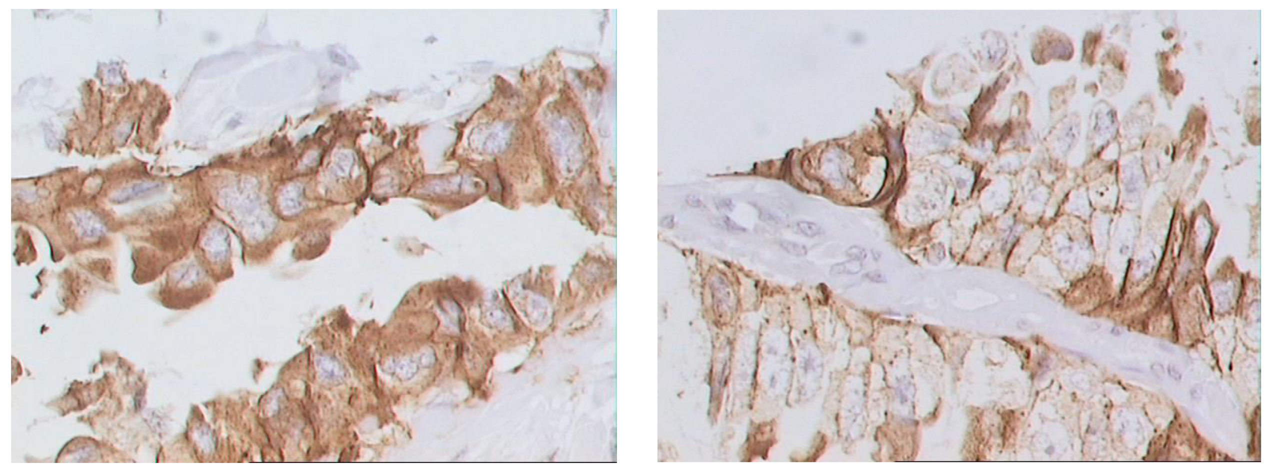


Figure 4. EGFR stained lung cancer, 40x images, coverslipped. Left: as received; Right: hematoxylin after fTAM shows ~50% cell nuclei captured. Stromal cells and nuclei unaffected (ROI 5 – 1.24 J/cm^2)

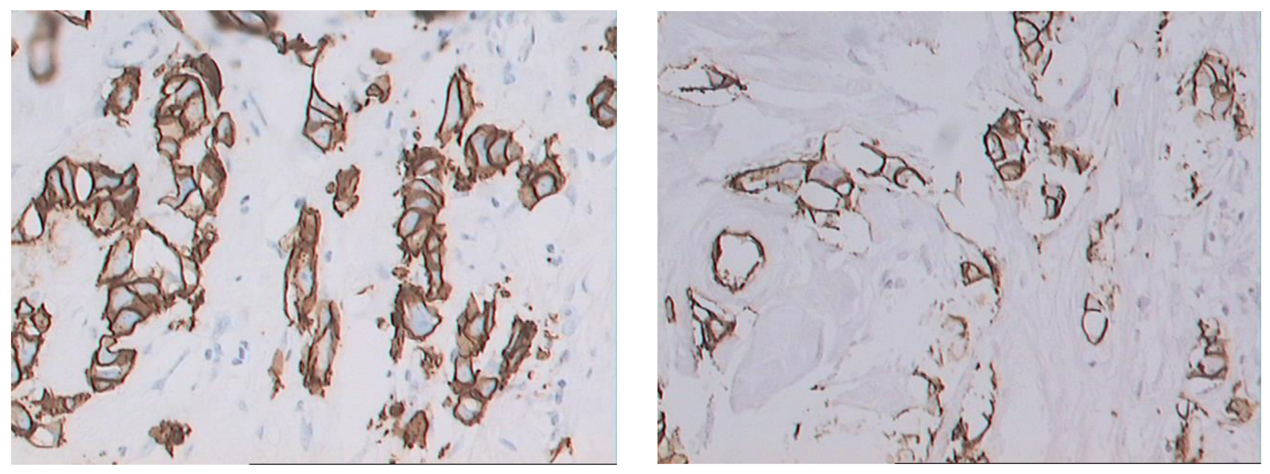


Figure 5. HER2neu stained breast cancer, 40x images, coverslipped. Left: as received; Right: with hematoxylin, after fTAM (ROI1 - 0.23 J/cm^2)

Conclusions

- fTAM system greatly increases the speed and simplicity of tissue microdissection without sacrificing specificity.
- The selected commercial 3M film with $\pm$1.5µm surface roughness effectively transmits vacuum in our slide holder, allowing the thermal contact necessary for fTAM microbonding of stained targets.
- Over a six-fold range in pulse fluence, the efficiency of microcapture increases but specificity remains high. Immunostained cells were captured while stromal cells were unaffected at all doses used.
- Nuclei of stained cells can be captured even when the stain target resides in the cell membrane (Her2neu and EGFR)
- Error of positioning the ROI mask is currently 250 $\pm$ 90 µm, limiting ROI selection to regions > 0.5 mm

Future Work

- Quantitative analysis of molecules extracted by fTAM should further guide optimization of dosimetry and tissue processing.
- Ratio of dsDNA to captured cell volume would determine nuclear capture efficiency with cell membrane receptor immunostaining.
- Downstream molecular analyses need to be optimized for analysis of fTAM clinical samples (e.g., RNA-Seq for gene expression)

References

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3. Bonner RF, Pohida TJ, et al. US Patent **7,695,752** April 13, 2010 Target-activated microtransfer. Assignee DHHS.
4. Buck MR, et al. US Patent **7,709,047** May 4, 2010 Target-activated microdissection. Assignee DHHS.

Acknowledgment

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